WHAT FACTORS DETERMINE THROMBOGENICITY?*

LEO VROMAN, Ph.D.

Principal Investigator, Interface Laboratory
Veterans Administration Hospital
Brooklyn, N.Y.

QUANTITATIVE analysis forces us to note only what can be expressed in numbers, and to compare events on this very narrow basis. Arranging a pinhole, a lightmeter, and a recorder properly, we can express our entire world as a single value, plotted to give us little more than the approximate time of day. We are committing the same injustice to reality if we describe someone only in pounds, or his endothelium only in terms of surface charge, critical surface tension, or ability to disturb flow.

Efforts to express thrombogenicity by *net electric charge* on the basis of *in vivo* experiments[1, 2] should take into account that even small applied voltages may damage the endothelium, causing thrombosis non-specifically.[3, 4] Positively charged materials do appear more thrombogenic,[5] but the activation of the Hageman factor (factor XII) requires a negatively charged surface, on which positively charged adsorbates are inhibitory.[6] The zeta potential of many solids will approach zero upon exposure to blood[9] because plasma will deposit protein on all surfaces, whether their charge is positive, negative,[7, 8] or zero.[14]

The other single popular value, *critical surface tension*, is a very sensitive shallow probe of molecular and submolecular solid surface structures that penetrates less than 24 Å,[10] yet effects from below upon the behavior of this outmost surface toward proteins may be profound.[11] On high energy surfaces, simple wettability for water serves perhaps as a measure either of cleanliness per se or of the cleaning method used; both wettability and definition of a "clean" gold surface have been disputed among experts.[12, 13] Others, less equipped, cannot hope to handle, and especially to implant, materials under better than these disputable conditions. The wettability of high energy surfaces is lowered

*Presented as part of a *Symposium on Problems in Evaluating the Blood Compatibility of Biomaterials* held by the Section on Basic Medical Sciences and the Section on Biomedical Engineering of the New York Academy of Medicine February 18 and 19, 1971.

immediately when proteins are adsorbed.[14, 15] On low energy (hydrophobic) surfaces, adsorbed proteins cause an increase in wettability;[14] here, perhaps, the molecules retain their native globular form most.[16] Correlation between wettability or work of adhesion and the effect on clotting are not perfect[17] and may be disturbed by the presence of air interfaces.[18] The complex interactions that relate clotting rates to surface properties have been reviewed recently.[19, 20] Interfaces participate normally in several coagulation reactions (Figure 1). We may assume that surface charge and wettability of these interfaces are important properties, but since they are among the first to change completely when proteins are adsorbed on them, there must be some other surface properties, now provided by the adsorbed matter, that determine subsequent events. These new properties include enzymatic activity, of which both nature and amount are dictated by the original surface forces that are now masked and that have selected and oriented the adsorbed proteins. Events related to clotting will be complicated further on materials to which some anticoagulant is more or less firmly bound. The behavior of purified proteins in solution at such or at any other interfaces[21] is no indication of events that occur in contact with the hundreds of species of protein present in whole plasma. The latter can be surmised from changes created in plasma by exposing it to large surface areas (powders); many wettable ones remove activity of factors II, VII, IX, and XII, while hydrophobic ones remove relatively more of factors V, VIII, and XI.[22] Heparinized powder surfaces reduce the activity of factor IX and, sometimes, of factor XI.[23]

None of these effects on clotting factors may be correlated very well with thrombogenicity.[24] As should be clear from other papers given at this symposium so far, the behavior of platelets from which thrombi will grow may be quite unrelated to the behavior of most clotting factors on the same surfaces. It is generally agreed that the adhesion of platelets follows the adsorption of some protein: namely globulins, according to some authors,[25] and fibrinogen, according to others.[26, 27] The preference of platelets for both collagen- and fibrinogen-coated surfaces may very well be a consequence of water structure acting as an entropic trigger.[28] If so, however, platelets should be expected to adhere only on fibrous molecules adsorbed in their native state, yet some authors think they adhere less to films of native protein.[29]

In our studies, proteins adsorbed on both high and low energy

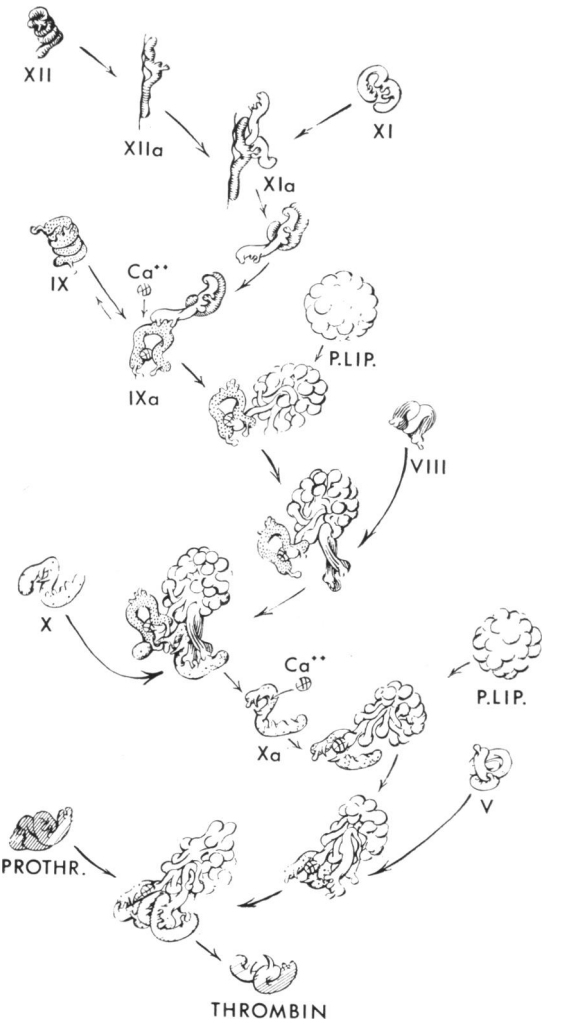

Fig. 1. Diagram of interactions among clotting factors, in which *a* indicates activation. Factor XII is activated by a solid surface; the activated factor (XII*a*) interacts with factor XI; some of the activation product activates factor IX in presence of calcium ions; factor IX*a* and factor VIII are adsorbed onto a phospholipid micelle, attract factor X and activate it; factor X*a*—in presence of calcium ions—and factor V are adsorbed onto a phospholipid micelle, attract, and convert prothrombin. Factors IX, X, and prothrombin may exist as a complex. Factors XI, VIII, and V, most easily removed by hydrophobic surfaces, may have hydrophobic groups most available for adsorption.

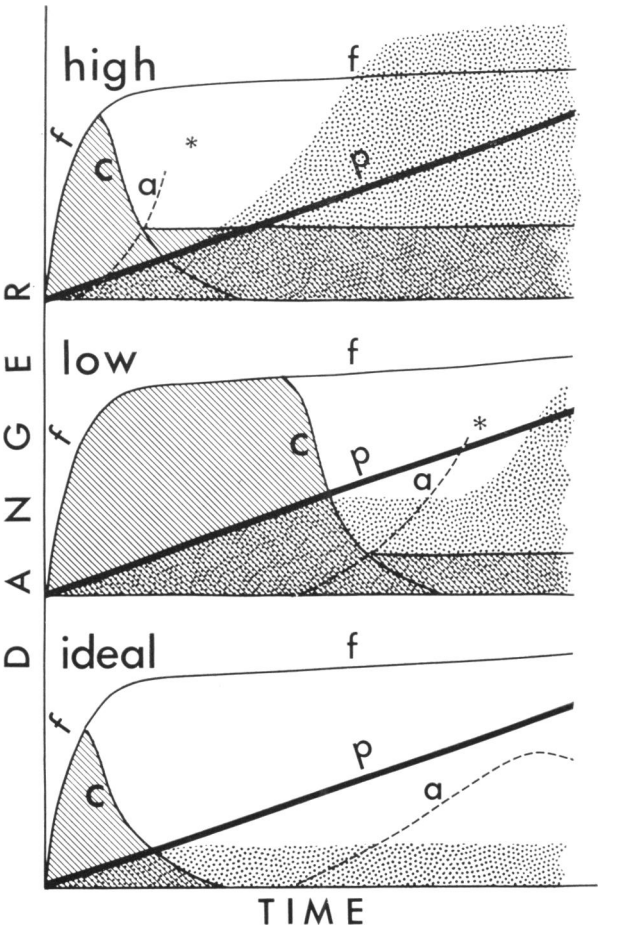

Fig. 2. Diagram of reaction sequence and its danger as it may be affected by certain surface properties, in which f = adsorption of fibrinogen; c = conversion of fibrinogen; a = activation of coagulation, resulting in * = thrombin formation; p = cumulative number of platelets colliding with the surface. Shaded areas indicate fibrinogen remaining at time indicated; stippled areas indicate platelets adhering to surface, here presumed to rise above those simply colliding once thrombin has formed. "High" and "low": events occurring at a high and at a low energy surface. "Ideal": presuming that the adsorption of fibrinogen cannot be avoided and that the resulting adhesion of more than one layer of platelets would be harmful, the ideal surface should allow early conversion of adsorbed fibrinogen and late activation of clotting.

surfaces were still able to react specifically with matching antisera[30] and may therefore be at least partially native. This has allowed us to identify proteins adsorbed out of plasma by *very* simple[31] as well as by more complex techniques.[32] Most helpful to the former was our finding that areas where matching antibody had been adsorbed onto

a preadsorbed protein film are most wettable and can be rendered visible by exposure to condensing water vapor. Adsorbed onto anodized tantalum-sputtered glass slides, protein films cause a shift in interference color (for example from deep tan to reddish purple), upon which the matching antiserum will deposit a layer to cause another color change (from reddish purple to blue violet).* Among more quantitative and complex approaches, the use of ellipsometer recordings showing both adsorption of plasma protein and subsequent antibodies has been most rewarding. Coagulation tests with deficient plasmas[33] and the observation of platelets suspended in deficient media and exposed to protein-coated surfaces further tempted us to draw the following preliminary conclusions (Figure 2):

1) Onto most surfaces, plasma deposits a monomolecular layer of fibrinogen within two seconds. Onto certain high-energy surfaces, factor XII activity is adsorbed more or less simultaneously and independently.

2) If the plasma is intact it so affects this fibrinogen film that it is no longer able to attract antifibrinogen. At lower plasma concentrations, greater activation, lower temperatures, and on lower-energy surfaces this alteration is slower. Under optimal conditions, it is completed within 10 seconds.

3) Platelets adhere preferentially to unconverted fibrinogen films.

4) Part of the remaining film is removed—or perhaps replaced by a thinner film—if intact factor XII is present in the bulk plasma.

5) Preadsorbed films of fibrinogen will lose their antigenicity when exposed to intact plasma and will lose thickness if the plasma contains intact factor XII, but not when the films are exposed to activated plasma, to albumin, or to 7S gamma globulins (Figure 3).

These findings indicate that the relation between activation of clotting and adhesion of platelets may be indirect enough to appear absent. The effect of fibrinogen on adhesion of platelets does not seem part of its function in clotting, and we have not been able to show any interactions between its adsorption and that of factor XII.[33] Thus far, surfaces that do not adsorb fibrinogen out of plasma in our laboratory also do not cause many platelets to adhere. For example, on one polymer (GMAC), heparin prevented adsorption of proteins (including fibrinogen) and adhesion of platelets, while on another polymer (TDMAC) it did not.[30] Preadsorbed films of albumin on anodized tantalum surfaces

*Slides showing such changes are available from Millis Research, Inc., Millis, Mass.

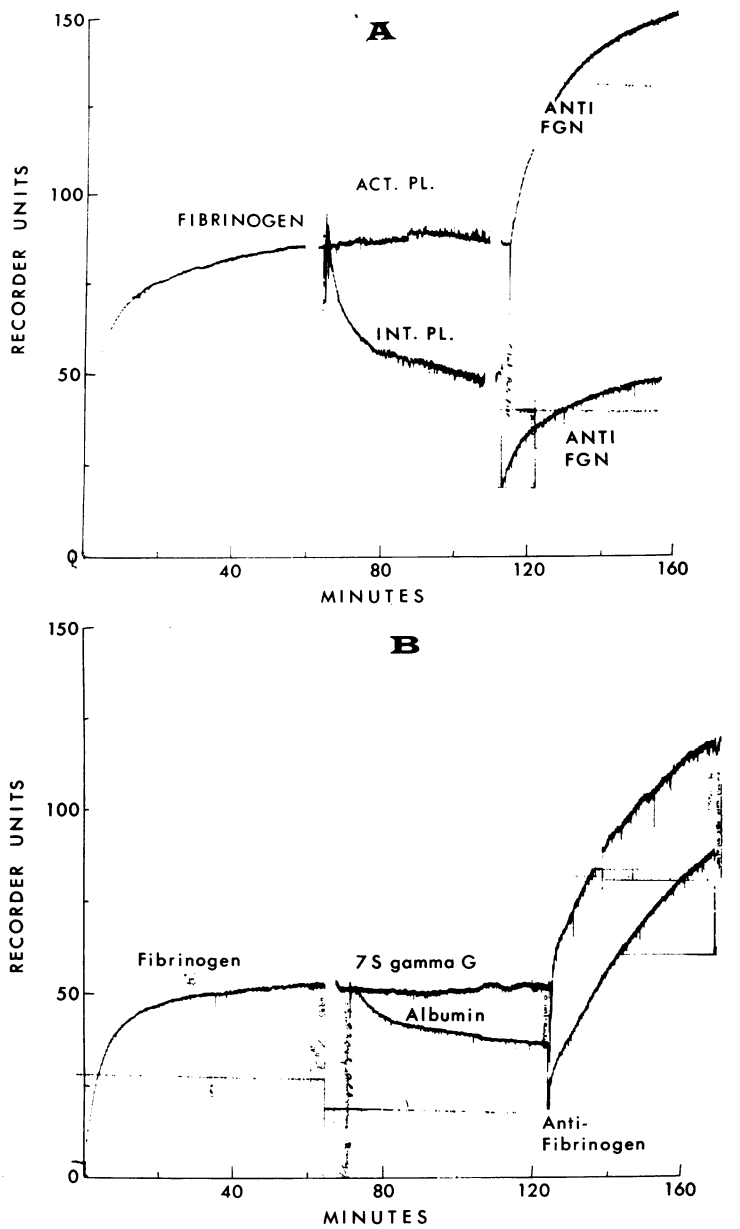

Fig. 3. Effect of: A) intact and activated normal plasma (int. pl. and act. pl.) and B) 7S gamma globulins and albumin, upon preadsorbed fibrinogen films. Ellipsometer recordings. An oxidized silicon crystal slice was used as reflecting solid. One recorder unit is equivalent to about 1 A. Human fibrinogen was adsorbed out of 15 ml. Veronal buffered saline, to which 0.4 ml. (1.2 mg.) of plasminogen-free fibrinogen was added at 0 time. Sixty minutes later solutions were replaced by 15 ml. of fresh buffer, readings were taken, and 0.1 ml. of plasma, or 0.2 ml. (0.4 mg.) of crystalline human albumin or 7S gamma globulins was added. Forty-five minutes later the contents of the cuvette were replaced again by buffer, readings were done, and 0.1 ml. of rabbit antihuman fibrinogen was added.

prevented adsorption of fibrinogen out of plasma and also prevented subsequent adhesion of platelets.

Yet even if this relation is established we must not conclude that it determines thrombogenicity, because: 1) in many experiments reported in this meeting, platelets are shown to start adhering long after adsorbed fibrinogen must have been converted, and 2) there may not be a clear relation at all between the thrombogenicity of a surface and its tendency to cover itself with a single uniform layer of platelets. It has been pointed out that thrombi do not form *in vivo* from uniformly distributed coatings but by a process resembling nucleation;[34] it can be initiated by a host of systemic as well as local changes.[35] If a thrombus forms at one point on some prosthetic device we may have to conclude that the device is thrombogenic but that its material is not, and that the focus of thrombus formation must have been a chemical or mechanical disturbance. On the arterial side, high flow rates may streamline focal platelet deposits and render the surface more uniform than it was before entering the bloodstream, erasing the faults created by manufacturing and handling.

These problems of contamination by handling, and of variabilities introduced by local flow *in vivo*, are discussed in other contributions to this symposium. For those who carry out *in vitro* studies of platelet deposition and thrombus formation during controlled flow, I must stress that the residence times of fibrinogen, of the agent that converts it, of whatever activation product that will stop this conversion, of platelets that collide with the changing surface, and of platelet-product release, all differ, and that all are probably essential factors in thrombogenesis. Therefore it should be rewarding to study effects of flow on the early, molecular interactions by subjecting a test surface to running platelet-poor plasma, and then to a stagnant platelet suspension. Preliminary experiments are now being carried out in our laboratory. For example, we squirted plasma onto a glass plate over which a buffer solution was flowing, then rinsed the plate with buffer followed by water, let it dry, exposed it to water vapor, and photographed the pattern created by adsorbed proteins; then we exposed the surface to antihuman fibrinogen serum, rinsed again with buffer and water, dried it once more, and exposed it again to water vapor. Since only the areas where antifibrinogen was adsorbed onto fibrinogen had now become increasingly wettable, the negative of a photograph taken at this time showed lighter areas

of fibrinogen rather than of converted fibrinogen or other adsorbate. The two photographs, superimposed by projecting them through a green and a red filter respectively, were then united to yield a color map of areas that should and others that should not be attractive to platelets. The demonstration suggests that one may identify proteins adsorbed out of plasma or blood; and we should hope to find their significance for platelet adhesion and thrombus formation under conditions of varied surface charge, surface energy and flow—provided the adsorbing solid is not a complex one. I believe that the papers by Dr. Hymie L. Nossel and by Drs. Michael B. Stemerman and T. H. Spaet as well as Dr. Salzman's review show clearly that very specific and complex interactions govern thrombosis and its prevention *in vivo*. The introduction of simpler surfaces may not lead to simpler events. The platelets "looking" at them must be regarded as cells, and cells do not react with their entire bodies to an averaged or bulk environment but with localized responses to localized environment features,[36] of which the diversified structure of water must be dominant.

REFERENCES

1. Sawyer, P. N., Srinivasan, S., Chopra, P. S., Martin, J. G., Lucas, T., Burrowes, C. B. and Sauvage, L.: Electrochemistry of thrombosis: An aid in the selection of prosthetic materials. *J. Biomed. Mater. Res. 4*:43, 1970.
2. Dawber, J. G. and Roberts, J. C.: An electrical double layer theory for platelet adhesiveness and initiation of intravascular thrombosis. *Thromb. Diath. Haemorrh. 29*:451, 1968.
3. Lamb, J. C., Isaacs, J. P., Bloom, W. L. and Harmer, D. S.: Electrical thrombosis of blood vessels: A voltage-dependent phenomenon. *Amer. J. Physiol. 208*:1006, 1965.
4. Martin, M. and Staubesand, J.: Intravasale Thrombusbildung durch elektrischen Strom und Thrombolyse. *Thromb. Diath. Haemorrh. 18*:570, 1967.
5. Milligan, H. L., Davis, J. and Edmark, K. W.: The search for correlation between electrokinetic phenomena and blood thrombus formation on implant materials. *J. Biomed. Mater. Res. 2*:51, 1968.
6. Nossel, H. L., Rubin, H., Drillings, M. and Hsieh, R.: *J. Clin. Invest. 47*:1172, 1968.
7. Nash, T., Allison, A. C., Harington, J. S.: Physico-chemical properties of silica in relation to its toxicity. *Nature 210*:259, 1966.
8. Messing, R. A.: Molecular inclusions. Adsorption of macromolecules on porous glass membranes. *J. Amer. Chem. Soc. 91*:2370, 1969.
9. Mirkovitch, V., Beck, R. E., Andrus, P. G. and Leininger, R. I.: The zeta potentials and blood compatibility characteristics of some selected solids. *J. Surg. Res. 4*:395, 1964.
10. Zissman, W. A.: Fundamental Factors in Detecting Chemicals as Adsorbed Films. In: *Surface Effects in Detection*, Bregman, J. I. and Dranieks, A., editors. Washington, D.C. Spartan, 1965, pp. 1-22.
11. Rothen, A.: The behavior of the tyrocidines at interfaces. *Biochim. Biophys. Acta 88*:606, 1964.
12. White, M. L.: The wetting of gold sur-

faces by water. *J. Phys. Chem. 68*:3083, 1964.
13. Bewig, K. W. and Zisman, W. A.: The wetting of gold and platinum by water. *J. Phys. Chem. 69*:4238, 1965.
14. Vroman, L.: Effect of adsorbed proteins on the wettability of hydrophilic and hydrophobic solids. *Nature 196*:476, 1962.
15. Baier, R. E. and Dutton, R. C.: Initial events in interactions of blood with a foreign surface. *J. Biomed. Mater. Res. 3*:191, 1969.
16. Brash, J. L. and Lyman, D. J.: Adsorption of plasma proteins in solution to uncharged hydrophobic polymer surfaces. *J. Biomed. Mater. Res. 3*:175, 1969.
17. Bischoff, K. B.: Discussion of correlations of blood coagulation with surface properties of materials. *J. Biomed. Mater. Res. 2*:89, 1968.
18. Maloney, J. V., Jr., Röher, D., Roth, E. and Latta, W. A.: The blood-prosthetic interface problem: the use of a hydrophilic protein gel to prevent blood clotting on prosthetic materials. *Surgery 66*:275, 1969.
19. Kingdon, H. S.: Evolution of enzyme activities and fibrin formation after contact of plasma with glass. *J. Biomed. Mater. Res. 3*:25, 1969.
20. Vroman, L., Adams, A. L. and Klings, M.: Interactions among human blood proteins at interfaces. *Fed. Proc. 30:* 1494, 1971.
21. Falb, R. D., Takahashi, M. T., Grode, G. A. and Leininger, R. I.: Studies on the stability and protein adsorption characteristics of heparinized polymer surfaces by radioisotope labeling techniques. *J. Biomed. Mater. Res. 1*:239, 1967.
22. Vroman, L.: Effects of hydrophobic surfaces upon blood coagulation. *Thromb. Diath. Haemorrh. 10*:455, 1964.
23. Oja, P. D., Holmes, G. W., Perkins, H. A. and Love, J.: Specific coagulation factor adsorption as related to functional groups on surfaces. *J. Biomed. Mater. Res. 3*:165, 1969.
24. Bokros, J. C., Gott, V. L., Lagrange, L. D., Fadali, A. M. and Ramos, M. D.: Heparin Sorptivity and blood compatibility of carbon surfaces. *J. Biomed. Mater. Res. 4*:145, 1970.
25. Packham, M. A., Evans, G., Glynn, M. F. and Mustard, J. F.: The effect of plasma proteins on the interaction of platelets with glass surfaces. *J. Lab. Clin. Med. 73*:698, 1969.
26. Zucker, M. B. and Vroman, L.: Platelet adhesion induced by fibrinogen adsorbed onto glass. *Proc. Soc. Exp. Biol. Med. 131*:318, 1969.
27. Salzman, E. W., Merrill, E. W., Binder, A., Wolf, C. F. W., Ashford, T. P. and Austen, W. G.: Protein-platelet interaction on heparinized surfaces. *J. Biomed. Mater. Res. 3*:69, 1969.
28. Abdulla, Y. H.: Effect of water structure on platelet aggregation *in vitro*. A theoretical and experimental study. *J. Atheroscler. Res. 7*:415, 1967.
29. Ziegler, T. F. and Miller, M. L.: Adsorption of fibrinogen on modified polytetrafluoroethylene surfaces. *J. Biomed. Mater. Res. 4*:259, 1970.
30. Vroman, L. and Adams, A. L.: Identification of rapid changes at plasma-solid interfaces. *J. Biomed. Mater. Res. 3*:43, 1969.
31. Vroman, L. and Adams, A. L.: Identification of adsorbed protein films by exposure to antisera and water vapor. *J. Biomed. Mater. Res. 3*:669, 1970.
32. Vroman, L. and Adams, A. L.: Findings with the recording ellipsometer suggesting rapid exchange of specific plasma proteins at liquid/solid interfaces. *Surface Sci. 16*:438, 1969.
33. Vroman, L. and Adams, A. L.: Peculiar behavior of blood at solid interfaces. *J. Polymer Sci. 34*:159, 1971.
34. Waugh, D. F. and Baughman, D. J.: Thrombin adsorption and possible relations to thrombus formation. *J. Biomed. Mater. Res. 3*:145, 1969.
35. Henry, R. L.: Methods for inducing experimental thrombosis. An annotated bibliography. *Angiol. 13*:554, 1962.
36. Weiss, P. A.: A cell is not an island entire of itself. *Perspectives Biol. Med. 14*:182, 1971.